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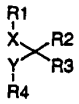

(I)

(57) Abstract

The present invention relates to compounds of Formula (I), and pharmaceutically acceptable salts or solvates thereof, or solvates of such salts, which compounds inhibit carboxypeptidase U and thus can be used in the prevention and treatment of diseases associated with carboxypeptidase U. In further aspects, the invention relates to compounds of the invention for use in therapy; to processes for preparation of such new compounds; to pharmaceutical compositions containing at least one compound of the invention, or a pharmaceutically acceptable salt or solvate thereof, as active ingredient; and to the use of the active compounds in the manufacture of medicaments for the medical use indicated above.

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NEW COMPOUNDS

FIELD OF THE INVENTION

The present invention relates to novel compounds, and pharmaceutically acceptable salts thereof, which inhibit basic carboxypeptidases, more specifically carboxypeptidase U, and thus can be used in the prevention and treatment of diseases wherein inhibition of carboxypeptidase U is beneficial. In further aspects, the invention relates to compounds of the invention for use in therapy; to processes for preparation of such new compounds; to pharmaceutical compositions containing at least one compound of the invention, or a pharmaceutically acceptable salt thereof, as active ingredient; and to the use of the active compounds in the manufacture of medicaments for the medical use indicated above.

BACKGROUND OF THE INVENTION

Fibrinolysis is the result of a series of enzymatic reactions resulting in the degradation of fibrin by plasmin. The activation of plasminogen is the central process in fibrinolysis. The cleavage of plasminogen to produce plasmin is accomplished by the plasminogen activators, tissue-type plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA). Initial plasmin degradation of fibrin generates carboxy-terminal lysine residues that serves as high affinity binding sites for plasminogen. Since plasminogen bound to fibrin is much more readily activated to plasmin than free plasminogen this mechanism provides a positive feedback regulation of fibrinolysis.

One of the endogenous inhibitors to fibrinolysis is carboxypeptidase U (CPU). CPU is also known as plasma carboxypeptidase B, active thrombin activatable fibrinolysis inhibitor (TAFIa), carboxypeptidase R and inducable carboxypeptidase activity. CPU is formed during coagulation and fibrinolysis from its precursor proCPU by the action of proteolytic enzymes *e.g.* thrombin, thrombin-thrombomodulin complex or plasmin. CPU cleaves basic amino acids at the carboxy-terminal of fibrin fragments. The loss of carboxy-terminal lysines and thereby of lysine binding sites for plasminogen then serves to inhibit fibrinolysis.

By inhibiting the loss of lysine binding sites for plasminogen and thus increase the rate of plasmin formation, effective inhibitors of carboxypeptidase U would be expected to facilitate fibrinolysis.

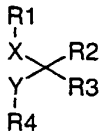
2-mercaptomethyl-3-guanidinoethylthiopropanoic acid is reported as a carboxypeptidase N inhibitor. More recently, this compound has been shown to inhibit CPU, Hendriks, D. *et al.*, Biochimica et Biophysica Acta, 1034 (1990) 86-92.

Guanidinoethylmercaptosuccinic acid is reported as a carboxypeptidase N inhibitor. More recently, this compound has been shown to inhibit CPU, Eaton, D. L., *et al.*, The Journal of Biological Chemistry. 266 (1991) 21833-21838.

DISCLOSURE OF THE INVENTION

It has surprisingly been found that compounds of the Formula I are particularly effective as inhibitors of carboxypeptidase U and thereby useful as medicaments for the treatment or prophylaxis of conditions wherein inhibition of carboxypeptidase U is beneficial.

In one aspect, the invention thus relates to compounds of the general Formula I,



(I)

or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

R_1 represents,

C_1-C_6 alkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;
heterocyclyl, containing at least one hetero atom selected from S or O,
and substituted with one or more basic groups such as amino, amidino and/or guanidino;
or aryl, substituted with one or more basic groups such as amino, amidino and/or guanidino,

R_2 represents H, acyl, acylamino, alkyl, alkylcarbamoyl, alkylthio, alkoxy, aroyl, aroylamino, aryloxy, arylthio, amidino, amino, aryl, carbamoyl, carboxy, cyano, cycloalkyl, formyl, guanidino, halogen, heterocyclyl, hydroxy, oxo, nitro, thiol, Z_2N-CO-O-, ZO-CO-NZ- or Z_2N-CO-NZ- group,

R_3 represents $COOR_5$, $SO(OR_5)$, SO_3R_5, $P{=}O(OR_5)_2$, $B(OR_5)_2$, $P{=}OR_5(OR_5)$, or tetrazole, or any carboxylic acid isostere,

R_4 represents SH, S-CO-C_1-C_6 alkyl or S-CO-aryl,

R_5 represents H, C_1-C_6 alkyl or aryl,

R_6 represents H or C_1-C_6 alkyl,

X represents O, S, SO, SO_2, $C(Z)_2$, N(Z), NR_6SO_2, SO_2NR_6, NR_6CO or $CONR_6$,

Y represents $C(Z)_2$,

Z represents independently H, C_1-C_6 alkyl, aryl, cycloalkyl or heterocyclyl.

Preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

R_1 represents,
cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;
heterocyclyl, containing at least one nitrogen atom;
heterocyclyl, containing at least one hetero atom selected from S or O, and substituted with one or more basic groups such as amino, amidino and/or guanidino;
or aryl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

R_2 represents H, acyl, acylamino, alkyl, alkylcarbamoyl, alkylthio, alkoxy, aroyl,

aroylamino, aryloxy, arylthio, amidino, amino, aryl, carbamoyl, carboxy, cyano, cycloalkyl, formyl, guanidino, halogen, heterocyclyl, hydroxy, oxo, nitro, thiol, Z_2N-CO-O-, ZO-CO-NZ- or Z_2N-CO-NZ- group,

R_3 represents $COOR_5$,

R_4 represents SH, S-CO-C_1-C_6 alkyl or S-CO-aryl,

R_5 represents H, C_1-C_6 alkyl or aryl,

R_6 represents H or C_1-C_6 alkyl,

X represents O, S, SO, SO_2, $C(Z)_2$, N(Z), NR_6SO_2, SO_2NR_6 or $CONR_6$,

Y represents $C(Z)_2$,

Z represents independently H, C_1-C_6 alkyl, aryl, cycloalkyl or heterocyclyl.

More preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

R_1 represents,

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

heterocyclyl, containing at least one hetero atom selected from S or O, and substituted with one or more basic groups such as amino, amidino and/or guanidino;

R_2 represents H, C_1-C_3 alkyl, amino, halogen or hydroxy,

R_3 represents $COOR_5$,

R_4 represents SH, S-CO-C_1-C_6 alkyl or S-CO-aryl,

R_5 represents H, C_1-C_6 alkyl or aryl,

X represents $C(Z)_2$,

Y represents $C(Z)_2$,

Z represents independently H or C_1-C_6 alkyl.

Even more preferred compounds according to the present invention are those of Formula I, or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

R_1 represents,

cycloalkyl, substituted with one or more basic groups such as amino, amidino and/or guanidino;

heterocyclyl, containing at least one nitrogen atom;

R_2 represents H, F, or C_1 alkyl,

R_3 represents $COOR_5$,

R_4 represents SH, S-CO-C_1-C_6 alkyl or S-CO-aryl,

R_5 represents H, C_1-C_6 alkyl or aryl,

X represents $C(Z)_2$,

Y represents $C(Z)_2$,

Z represents independently H or C_1-C_6 alkyl.

Most preferred compounds according to the present invention are those of Formula I or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt, wherein

R_1 represents cyclopentyl, pyridyl, pyrimidinyl, piperidinyl or thiazolyl,

R_2 represents H, F, or C_1 alkyl,

R_3 represents $COOR_5$,

R_4 represents SH,

R_5 represents H,

X represents CHZ,

Y represents CHZ,

Z represents independently H or C_1-C_6 alkyl.

The following definitions shall apply throughout the specification and the appended claims:

The term "basic group" denotes a basic group, wherein the conjugate acid of said basic group has a pKa of from about –5 to about 25, preferably of from 1 to 15.

The term "carboxylic acid isostere" denotes an acidic group having a pKa of from about –5 to about 25, preferably of from 1 to 15.

The term "C_1–C_6 alkyl" denotes a straight or branched, saturated or unsaturated, substituted or unsubstituted alkyl group having 1 to 6 carbon atoms in the chain wherein the alkyl group may optionally be interrupted by one or more heteroatoms selected from O, N or S. Examples of said alkyl include, but is not limited to, methyl, ethyl, ethenyl, ethynyl, n-propyl, iso-propyl, propenyl, iso-propenyl, propynyl, n-butyl, iso-butyl, sec-butyl, t-butyl, butenyl, iso-butenyl, butynyl and straight- and branched-chain pentyl and hexyl.

The term "C_1–C_3 alkyl" denotes a straight or branched, saturated or unsaturated, substituted or unsubstituted alkyl group having 1 to 3 carbon atoms in the chain wherein the alkyl group may optionally be interrupted by one or more heteroatoms selected from O, N or S. Examples of said alkyl include, but is not limited to, methyl, ethyl, ethenyl, ethynyl, n-propyl, iso-propyl, propenyl, iso-propenyl, propynyl.

The term "C_1 alkyl" denotes a substituted or unsubstituted alkyl group having 1 carbon atom. An example of said alkyl include, but is not limited to, methyl,

The term "C_1-C_6 alkoxy" denotes an alkyl-O-group, wherein C_1-C_6 alkyl is as defined above.

The term "C_1-C_3 alkoxy" denotes an alkyl-O-group, wherein C_1-C_3 alkyl is as defined above.

The term "heterocyclyl" denotes a substituted or unsubstituted, 4- to 10- membered monocyclic or multicyclic ring system in which one or more of the atoms in the ring or rings is an element other than carbon, for example nitrogen, oxygen or sulfur, especially 4-, 5- or 6-membered aromatic or alifatic hetorocyclic groups, and includes, but is not limited to azetidine, furan, thiophene, pyrrole, pyrroline, pyrrolidine, dioxolane, oxathiolane, oxazolane, oxazole, thiazole, imidazole, imidazoline, imidazolidine, pyrazole, pyrazoline, pyrazolidine, isoxazole, isothiazole, oxadiazole, furazan, triazole, thiadiazole, pyran, pyridine, piperidine, dioxane, morpholine, dithiane, oxathiane, thiomorpholine, pyridazine, pyrimidine, pyrazine, piperazine, triazine, thiadiazine, dithiazine, azaindole, azaindoline,

indole, indoline, naphthyridine groups, and shall be understood to include all isomers of the above identified groups. The term "azetidinyl" shall for example by understood to include the 2-, and 3-isomers and the terms "pyridyl" and "piperidinyl" shall for example by understood to include the 2-, 3-, and 4-isomers.

The term "cycloalkyl" denotes a saturated or unsaturated, substituted or unsubstituted, non-aromatic ring composed of 3, 4, 5, 6 or 7 carbon atoms, and includes, but is not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooheptyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclopentadienyl, cyclohexadienyl and cycloheptadienyl groups,

The term "halogen" includes fluoro, chloro, bromo and iodo groups.

The term "aryl" denotes a substituted or unsubstituted C_6-C_{14} aromatic hydrocarbon and includes, but is not limited to, phenyl, naphthyl, indenyl, antracenyl, fenantrenyl, and fluorenyl.

The term "aryloxy" denotes an aryl-O-group, wherein aryl is as defined above.

The term "acyl" denotes an alkyl-CO-group, wherein alkyl is as defined above.

The term "aroyl" denotes an aryl-CO-group, wherein aryl is as defined above.

The term "alkylthio" denotes an alkyl-S-group, wherein alkyl is as defined above.

The term "arylthio" denotes an aryl-S-group, wherein aryl is as defined above.

The term "aroylamino" denotes an aroyl-N(Z)-group, wherein aroyl and Z is as defined above.

The term "acylamino" denotes an acyl-N(Z)-group, wherein acyl and Z is as defined above.

The term "carbamoyl" denotes a H_2N-CO-group.

The term "alkylcarbamoyl" denotes a Z_2N-CO-group wherein Z is as defined above.

The term "substituted" denotes an "C_1 alkyl", "C_1-C_3 alkyl", "C_1-C_6 alkyl", "cycloalkyl", "heterocyclyl" or a "aryl" group as defined above which is substituted by one or more acyl, acylamino, alkyl, alkylcarbamoyl, alkylthio, alkoxy, aroyl, aroylamino, aryloxy, arylthio, amidino, amino, aryl, carbamoyl, carboxy, cyano, cycloalkyl, formyl, guanidino, halogen, heterocyclyl, hydroxy, oxo, nitro, thiol, thio, Z_2N-CO-O-, ZO-CO-NZ-, or Z_2N-CO-NZ-groups.

Moreover, the compounds of Formula I wherein R_4 is mercapto may be present in the form of a dimer which is bonded via –S-S-bond, which is also included in this invention.

Both the pure enantiomers, racemic mixtures and unequal mixtures of two enantiomers are within the scope of the present invention. It should also be understood that all the diastereomeric forms possible are within the scope of the invention. Also included in the invention are derivatives of the compounds of the Formula I which have the biological function of the compounds of Formula I, such as prodrugs.

Depending on the process conditions the compounds of Formula I are obtained either in neutral or salt form or as a solvate, e.g. a hydrate, and are all within the scope of the present invention.

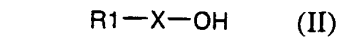
Preparation

The present invention also provides the process A-C for the manufacture of compounds with the general Formula I.

Process A

Process A for manufacture of compounds with the general Formula I, wherein R_1, R_3, R_4, and Y are as defined above and R_2 is H, and X is $C(Z)_2$, comprises the following steps:

a) Compounds of the general Formula II,



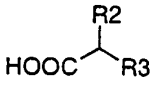
(II)

wherein R_1, is as defined for Formula I and X is $C(Z)_2$,which are either commercially available or are available using known techniques, can be converted into a compound of the general Formula III,

(III)

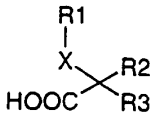
wherein L is a suitable leaving group, such as chloro, bromo, iodo, triflate or tosyl, under standard conditions using a suitable reagent, such as PPh_3/CBr_4, TosCl/pyridine or $(CF_3SO_2)_2O$/TEA.

b) Compounds of the general Formula III can thereafter be reacted with compounds of the general Formula IV,



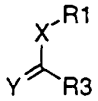
(IV)

wherein R_2 and R_3 are as defined for Formula I, which are either commercially available, or are available using known techniques, in the presence of a suitable base, such as K_2CO_3 or NaH, under standard conditions to give compounds of the general Formula V.



(V)

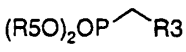
c) Compounds of the general Formula V wherein R_1 and R_3 are as defined for Formula I and X is $C(Z)_2$ and R_2 is H can thereafter be converted to compounds of the general Formula VI,



(VI)

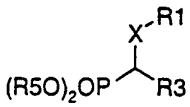
by treatment with formaldehyd in the presence of a suitable base, such as Et_2NH, under standard conditions.

d) Compounds of the general Formula VI can also be prepared by treating compounds of the general Formula VII,



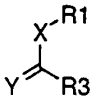
(VII)

wherein R_3 and R_5 are as defined for Formula I, with an alkylating agent of the general Formula III in the presence of a suitable base, such as LDA or NaH, under standard conditions to give compounds of the general Formula VIII,



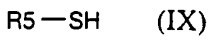
(VIII)

e) Compounds of the general Formula VIII can thereafter be reacted with an appropriate aldehyde or ketone $OC(Z)_2$, in the presence of a suitable base, such as KOtBu, LDA or NaH, under standard conditions to give a compound of the general Formula VI.



(VI)

f) Compounds of the general Formula VI can be further reacted with compounds of the general Formula IX,



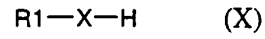
(IX)

wherein R_5 is a suitable protecting group, such as Ac, Bz, PMB or Bn, alone or in the presence of a suitable base, such as NaOMe, NaH or triethylamine or alternatively in the presence of a free-radical initiator, such as AIBN under standard conditions to give compounds of the general Formula I, wherein R_1, R_3, R_4, and Y are as defined for Formula I and R_2 is H and X is $C(Z)_2$.

Process B

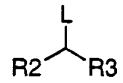
Process B for manufacture of compounds with the general Formula I, wherein R_1, R_2, R_3, and R_4, are as defined in Formula I and Y is CH_2, and X is O, S, SO, SO_2, $C(Z)_2$, or N(Z), comprises the following steps:

a) Reacting a compound of the general Formula X,



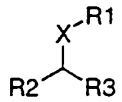
(X)

wherein R_1 is as defined for Formula I and X is O, S, or N(Z), with an alkylating agent of the general Formula XI,



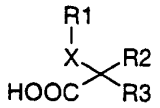
(XI)

wherein R_2 and R_3 are as defined for Formula I and L is a suitable leaving group, such as a chloro, bromo, iodo, triflate or tosylate group, under standard conditions using suitable reagents, such as NaH, Ag_2CO_3, or Bu_4NHSO_4/NaOH, to give compounds of the general Formula XII,



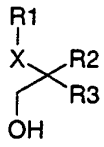
(XII)

b) Compounds of the general Formula XII can thereafter be reacted with carbon dioxide in the presence of a suitable base, such as LDA or KHMDS under standard conditions to give a compound of the general Formula XIII,



(XIII)

(c) Compounds of the general Formula XIII can thereafter be reacted with an alkyl chloroformate, such as ClCOOMe in the presence of a base, such as triethylamine, and thereafter reducing the formed mixed anhydride with a suitable reducing agent, such as $NaBH_4$, under standard conditions, to give a compound of the general Formula XIV



(XIV)

(d) Compounds of the general Formula XIV may thereafter be reacted with a compound of the general Formula IX

R5—SH (IX)

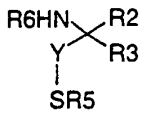
wherein R_5 is a suitable protecting group, such as Ac or Bz, in the presence of a suitable reagent, such as PPh_3/DIAD, under standard conditions to give compounds of the general Formula I, wherein R_1, R_2, R_3, and R_4 are as defined above and Y is CH_2 and X is O, S, $C(Z)_2$, or N(Z).

e) Compounds of the general Formula I, wherein R_1, R_2, R_3, R_4 and Y are as defined above and and X is S may thereafter be reacted with a suitable oxidizing reagent, such as MCPBA under standard conditions to give compounds of the general Formula I, wherein R_1, R_2, R_3, R_4 and Y are as defined above and and X is SO or SO_2.

Process C

Process C for manufacture of compounds with the general Formula I, wherein R_1, R_2, R_3, R_4, and Y, are as defined above and X is NR_6CO, $CONR_6$, SO_2NR_6 or NR_6SO_2 comprises the following steps:

a) Reacting a compound of the general Formula XV,



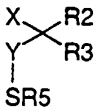
(XV)

wherein R_2, R_3, R_6 and Y are as defined for Formula I and R_5 is a suitable protecting group, such as Ac, Bz, PMB or Bn, with a compound of the general Formula XVI,

R1–X (XVI)

wherein R_1 is as defined for Formula I and X is COOH or SO_2Cl in the presence of suitable coupling reagents, such as PyBOP/DIPEA, DCC/HOBt, EDC/TEA/DMAP or pyridine under standard conditions to give compounds of the general Formula I, wherein R_1, R_2, R_3, R_4, and Y, are as defined above and X is NR_6CO or NR_6SO_2.

b) Reacting a compound of the general Formula XVII,



(XVII)

wherein R_2, R_3, and Y are as defined for Formula I and X is COOH or SO_2Cl and R_5 is a suitable protecting group, such as Ac, Bz, PMB or Bn, with a compound of the general Formula XVIII,

$R6–NH_2$ (XVIII)

wherein R_6 is as defined for Formula I in the presence of suitable coupling reagents, such as PyBOP/DIPEA, DCC/HOBt, EDC/TEA/DMAP or pyridine under standard conditions to give compounds of the general Formula I, wherein R_1, R_2, R_3, R_4 and Y are as defined above and X is $CONR_6$ or SO_2NR_6.

It will be appreciated by those skilled in the art that in the processes described above the functional groups of intermediate compounds may need to be protected by suitable protecting groups.

Functional groups, which it is desirable to protect, include hydroxy, amino, mercapto and carboxylic acid. Suitable protecting groups for hydroxy include trialkylsilyl or diarylalkylsilyl (e.g. t-butyldimethylsilyl, t-butyldiphenylsilyl or trimethylsilyl), tetrahydropyranyl and benzyl. Suitable protecting groups for amino, amidino and guanidino include t-butyloxycarbonyl and benzyloxy-carbonyl. Suitable protecting groups for mercapto include $CO\text{-}C_{1\text{-}6}$ alkyl, p-methoxybenzyl and trityl. Suitable protecting groups for carboxylic acid include $C_{1\text{-}6}$ alkyl and benzyl esters.

Protecting groups may be removed in accordance with techniques, which are well known to those skilled in the art and as described hereinafter.

Certain protected derivatives of compounds of Formula I, which may be made prior to a final deprotection stage to form compounds of Formula I, are novel.

The use of protecting groups is described in 'Protective Groups in Organic Synthesis', 2nd edition, T.W. Greene & P.G.M. Wutz, Wiley-Interscience (1991). The protective group may also be a polymer resin such as Wang resin or a 2-chorotrityl chloride resin.

It will also be appreciated by those skilled in the art, although such protected derivatives of compounds of Formula I may not possess pharmacological activity as such, they may be administered parenterally or orally and thereafter metabolised in the body to form compounds of the invention which are pharmacologically active. Such derivatives may

therefore be described as "prodrugs". All prodrugs of compounds of Formula I are included within the scope of the invention.

It should also be understood that all polymorphs, amorphous forms, anhydrates, hydrates, solvates of the compounds of the present invention are within the scope of the invention.

Pharmaceutical formulations

In yet a further aspect, the invention relates to pharmaceutical compositions containing at least one compound of the present invention, or a pharmaceutically acceptable salt thereof, as active ingredient.

For clinical use, the compounds of the invention are formulated into pharmaceutical formu-lations for oral, intravenous, subcutaneous, tracheal, bronchial, intranasal, pulmonary, transdermal, buccal, rectal, parenteral or other mode of administration. The pharmaceutical formulation contains a compound of the invention in combination with one or more pharmaceutically acceptable ingredients. The carrier may be in the form of a solid, semi-solid or liquid diluent, or a capsule. These pharmaceutical preparations are a further object of the invention. Usually the amount of active compounds is between 0.1–95% by weight of the preparation.

In the preparation of pharmaceutical formulations containing a compound of the present invention the compound selected may be mixed with solid, powdered ingredients, such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives, gelatin, or another suitable ingredient, as well as with disintegrating agents and lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylene glycol waxes. The mixture may then be processed into granules or pressed into tablets.

Soft gelatine capsules may be prepared with capsules containing a mixture of the active compound or compounds of the invention, vegetable oil, fat, or other suitable vehicle for soft gelatine capsules. Hard gelatine capsules may contain granules of the active compound. Hard gelatine capsules may also contain the active compound in combination

with solid powdered ingredients such as lactose, saccharose, sorbitol, mannitol, potato starch, cornstarch, amylopectin, cellulose derivatives or gelatine.

Dosage units for rectal administration may be prepared (i) in the form of suppositories which contain the active substance mixed with a neutral fat base; (ii) in the form of a gelatine rectal capsule which contains the active substance in a mixture with a vegetable oil, paraffin oil or other suitable vehicle for gelatine rectal capsules; (iii) in the form of a ready-made micro enema; or (iv) in the form of a dry micro enema formulation to be reconstituted in a suitable solvent just prior to administration.

Liquid preparations may be prepared in the form of syrups or suspensions, e.g. solutions or suspensions containing the active ingredient and the remainder consisting, for example, of sugar or sugar alcohols and a mixture of ethanol, water, glycerol, propylene glycol and polyethylene glycol. If desired, such liquid preparations may contain colouring agents, flavouring agents, preservatives, saccharine and carboxymethyl cellulose or other thickening agents. Liquid preparations may also be prepared in the form of a dry powder to be reconstituted with a suitable solvent prior to use.

Solutions for parenteral administration may be prepared as a solution of a compound of the invention in a pharmaceutically acceptable solvent. These solutions may also contain stabilizing ingredients, preservatives and/or buffering ingredients. Solutions for parenteral administration may also be prepared as a dry preparation to by reconstituted with a suitable solvent before use.

The typical daily dose of the active substance varies within a wide range and will depend on various factors such as for example the individual requirement of each patient, the route of administration and the disease. In general, oral and parenteral dosages will be in the range of 0.1 to 1000 mg per day of active substance.

Medical and pharmaceutical use

The compounds of the invention are inhibitors of carboxypeptidase U either as such or, in the case of prodrugs, after administration. The compounds of the invention are thus expected to be useful in those conditions where inhibition of carboxypeptidase U is beneficial, such as in the treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues of mammals, including man.

It is known that hypercoagulability may lead to thrombo-embolic diseases. Conditions associated with hypercoagulability and thrombo-embolic diseases which may be mentioned include protein C resistance and inherited or aquired deficiences in antithrombin III, protein C, protein S and heparin cofactor II. Other conditions known to be associated with hyper-coagulability and thrombo-embolic disease include circulatory and septic shock, circulating antiphospholipid antibodies, homocysteinami, heparin induced thrombocytopenia and defects in fibrinolysis. The compounds of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions. The compounds of the invention are further indicated in the treatment of conditions where there is an undesirable excess of proCPU/CPU.

Particular disease states which may be mentioned include the therapeutic and /or prophylactic treatment of venous thrombosis and pulmonary embolism, arterial thrombosis (e.g. in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis) and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricle after transmural myocardial infarction.

Moreover, the compounds of the invention are expected to have utility in prophylaxis of re-occlusion and restenosis (*i.e.* thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other

mechanism, fibrinolytic treatment when blood is in contact with foreign surfaces in the body, such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device, and fibrinolytic treatment when blood is in contact with medical devices outside the body, such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

The compounds of the invention may also be combined and/or coadministered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P_2T) antagonists and thrombin inhibitors.

The compounds of the invention may further be combined and/or coadministered with thrombolytics such as tissue plasminogen activator (natural, recombinant or modified), streptokinase, urokinase, prourokinase, anisoylated plasminogen-streptokinase activator complex (APSAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction and stroke.

In vitro experiments

The inhibiting effect of the compounds of the present invention was estimated using the assay described in: Dirk Hendriks, Simon Scharpé and Marc van Sande, Clinical Chemistry, 31, 1936-1939 (1985); and Wei Wang, Dirk F. Hendriks, Simon S. Scharpé, The Journal of Biological Chemistry, 269, 15937-15944 (1994).

EXAMPLES

General Experimental Procedures

Mass spectra were recorded on a Finnigan MAT TSQ 700 triple quadropole mass spectrometer equipped with an electrospray interface (FAB-MS) and VG Platform II mass spectrometer equipped with an electrospray interface (LC-MS). ^{1}H NMR and ^{13}C NMR measurements were performed on Varian UNITY plus 400, 500 and 600 spectrometers, operating at ^{1}H frequencies of 400, 500 and 600 MHz respectively. Chemical shifts are

given in ppm with the solvent as internal standard. Organic extracts were dried using $MgSO_4$ or Na_2SO_4 as the drying agent. Chromatography separations were performed using Merck Silica gel 60 (0.063-0.200 mm). HPLC separations were performed on a HIGHCROM KR100-10C8 column.

Example 1

2-Mercaptomethyl-3-piperidin-4-yl-propionic acid

(a) 3-Piperidin-4-yl-propionic acid

A solution of 3-pyridin-4-yl-acrylic acid (4.20 g, 28.0 mmol) in water (50 mL) and ammonia (aq, 25 %, 4 mL) was hydrogenated at 60 bar in a high pressure steel autoclave in presence of ruthenium (5 % on alumina, 439 mg). When hydrogen pressure remained constant (3 days) the catalyst was removed from the reaction mixture by filtration. The catalyst was washed with ethanol and water, and the ethanol was removed from the solution on a rotavapor and the aqueous solution was freeze dried to give 3-piperidin-4-yl-propionic acid (4.30 g, 100 %).

(b) 4-(2-carboxy-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester

A solution of 3-piperidin-4-yl-propionic acid (4.79 g, 30.5 mmol), di-*tert*-butyl-dicarbonate (6.98 g, 32.0 mmol) and $NaHCO_3$ (2.69 g, 32.0 mmol) in THF/water (1:1, 50 mL) were stirred at room temperature for 22 h. Another portion of di-*tert*-butyl-dicarbonate (2.00 g, 9.10 mmol) was added together with a catalytic amount of DMAP, the resulting mixture was stirred for another four days. THF was removed under reduced pressure and the aqueous phase was extracted with CH_2Cl_2. The aqueous was then acidified to pH 2 with 1M HCl and the acid extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried and concentrated *in vacuo* to yield 4-(2-carboxy-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester as a white solid (6.36 g, 81 %).

(c) 4-(3-Benzylsulfanyl-2-carboxy-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester

BuLi (1.6 M, 15.3 mL, 24.4 mmol) was added to a solution of diisopropylamine (3.43 mL, 24.4 mmol) in THF (3 mL) at –78°C under argon. After a few min the solution was allowed to warm up to room temperature over a period of 15 min. The resulting LDA

solution was slowly added to a solution of 4-(2-carboxy-ethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (3.07 g, 11.9 mmol) in THF (7 mL) at –78°C. The resulting solution was stirred at –78°C for 10 min., THF (20 mL) was added during that time in order to dissolve the anion which had solidified. The dianion was cooled to –78°C and bromomethyl thiobenzylether (2.72 g, 12.5 mmol) was added as a solution in THF (3 mL), the solution was stirred at –78°C for 30 min, at 0°C for 30 min and then allowed to warm up to room temperature and stirred overnight. The reaction mixture was acidified with 1 M HCl, diluted with EtOAc and the organic phase was washed with water and dried. The crude product was purified by flash chromatography (MeOH/$CHCl_3$,1:9) to yield 4-(3-Benzylsulfanyl-2-carboxy-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester as a pale yellow oil (3.12 g, 66 %).

(d) 4-(2-Carboxy-3-mercapto-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester

Sodium metal (513 mg, 22.5 mmol) was added in portions during 5 min. to a solution of 4-(3-Benzylsulfanyl-2-carboxy-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.9 g, 2.29 mmol) in THF (45 mL) and liquid ammonia (50 mL) at -60°C under argon. After stirring for 15 min. ammonium chloride (1.7 g, 31.5 mmol) was added in portions. The cooling bath was removed and the ammonia was evaporated using a stream of argon. 0.5 M NaOH was added and the mixture was washed with heptane. The aqueous phase was acidified with 2 M HCl and extracted with methylene chloride. The organic phase was washed with brine, dried and concentrated under reduced pressure to give 4-(2-Carboxy-3-mercapto-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.7 g, 100 %).

(e) 2-Mercaptomethyl-3-piperidin-4-yl-propionic acid

To a solution of 4-(2-Carboxy-3-mercapto-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.7 g, 2.29 mmol) in methylene chloride (8 mL) under argon was added triethyl-silane (731 µL, 4.58 mmol) followed by TFA (4 mL). The reaction mixture was stirred for 60 min. and then concentrated under reduced pressure. Purification by HPLC (10 → 30 % acetonitrile, 0.1 % TFA in water) gave the title compound as the TFA salt (447 mg, 61 %).

^{1}H NMR (400 MHz, D_2O): δ 1.34-1.50 (m, 2H), 1.54-1.76 (m, 3H), 1.90-1.99 (m, 1H), 2.0-2.1 (m, 1H), 2.9-3.05 (m, 5H), 3.38-3.48 (m, 2H).

MS (+) 204 (M+1).

Example 2

3-(1-Acetyl-piperidin-4-yl)-2-mercaptomethyl-propionic acid

A solution of 2-Mercaptomethyl-3-piperidin-4-yl-propionic acid TFA salt (0.1 g, 0.32 mmol) in acetic anhydride (2 mL) was stirred over night under argon and then concentrated under reduced pressure. Purification by HPLC (10 → 50 % acetonitrile, 0.1 % TFA in water) gave the title compound (63 mg, 80 %).

^{1}H NMR (500 MHz, CD_3OD): δ 1.0-1.22 (m, 2H), 1.46-1.55 (m, 1H), 1.55-1.66 (m, 2H), 1.67-1.79 (m, 1H), 1.81-1.92 (m, 1H), 2.08 (s, 3H), 2.55-2.73 (m, 4H), 3.03-3.12 (m, 1H), 3.85-3.94 (m, 1H), 4.45-4.53 (m, 1H).
MS (+) 246 (M+1).

Example 3

3-Mercapto-5-methyl-2-piperidin-4-ylmethyl-hexanoic acid

(a) 4-Hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester

To a solution of , 4-(hydroxymethyl)piperidine (5.00 g, 43.41 mmol THF/ H_2O (1:1, 120 mL) was added di-*t*-butyl dicarbonate (9.47 g, 43.41 mmol). The reaction mixture was stirred for 16 h at room temperature. The reaction mixture was then poured into H_2O (500 mL) and extracted with ethyl acetate (3 x 250 mL). The organic layers were combined and washed with water. The organic layer was dried over sodium sulfate, filtered, and then concentrated under reduced pressure to give 4-hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (9.01 g, 96%).

(b) 4-Bromomethyl-piperidine-1-carboxylic acid *tert*-butyl ester

To a solution 4-hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (8.75 g, 40.64 mmol) in diethyl ether (200 mL) at 0^0C under nitrogen. were added triphenyl phosphine (21.32 g, 81.28 mmol) and carbon tetrabromide (26.96 g, 81.28 mmol). The mixture was allowed to warm to room temperature and stirred under nitrogen for 48 h. The reaction mixture was filtered through a pad of Celite and the organic filtrate was washed with 5 % NaS_2O_3, water, brine, and dried. The mixture was filtered and concentrated under reduced

pressure. The crude product was purified by column chromatography (ethyl acetate/hexane, 1:9) to give 4-bromomethyl-piperidine-1-carboxylic acid *tert*-butyl ester (8.26 g, 73 %).

(c) 4-[2-*tert*-Butoxycarbonyl-2-(diethoxy-phosphoryl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester

tert-Butyl diethylphosphonoacetate (75.0 g, 297.32 mmol) was added dropwise to a suspension of sodium hydride (8.03 g, 334.58 mmol) in DMF (450 mL) at 0^0C under nitrogen. The mixture was stirred at 0^0C for 0.5 h and at room temperature for 0.5 h. 4-bromomethyl-piperidine-1-carboxylic acid *tert*-butyl ester (20.68 g, 74.34 mmol) in DMF (50 mL) was added dropwise to the reaction mixture and the reaction was heated to 60^0C and stirred for 16 h. The reaction was cooled to room temperature, poured into H_2O and extracted with ethyl acetate. The organic layers were combined and washed with water. The organic layer was dried, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/hexane, 3:7) to give 4-[2-*tert*-Butoxycarbonyl-2-(diethoxy-phosphoryl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester and unreacted *t*-butyl diethylphosphonoacetate. The product was 47 % pure by HPLC. The product was further purified by vacuum distillation to give 77 % purity. This mixture was taken on to the next reaction.

(d) 4-(2-*tert*-Butoxycarbonyl-5-methyl-hex-2-enyl)-piperidine-1-carboxylic acid *tert*-butyl ester

4-[2-*tert*-Butoxycarbonyl-2-(diethoxy-phosphoryl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester(8.1 g) in 20 mL DME was added dropwise to a suspension of sodium hydride (1.04 g, 43.13 mmol) in DME (20 mL) at 0^0C under nitrogen. The mixture was stirred for 0.75 h and isovaleraldehyde (7.76 g, 90.1 mmol) was added dropwise to the mixture. The mixture was allowed to warm to room temperature and stirred for 48 h. The mixture was diluted with ether and washed with water. The organic layer was dried, filtered, and concentrated under reduced pressure to give 9.4 g of a yellow oil. The crude product was purified by column chromatography (ethyl acetate/hexane, 1:50) to give 4-(2-*tert*-butoxycarbonyl-5-methyl-hex-2-enyl)-piperidine-1-carboxylic acid *tert*-butyl ester (1.53 g, 24 %) for two reactions.

(e) 4-[2-*tert*-Butoxycarbonyl-3-(4-methoxy-benzylsulfanyl)-5-methyl-hexyl]-piperidine-1-carboxylic acid *tert*-butyl ester

A solution of 4-(2-*tert*-butoxycarbonyl-5-methyl-hex-2-enyl)-piperidine-1-carboxylic acid *tert*-butyl ester (2.0 g, 5.24 mmol) in DMF (5 mL) was added to a mixture of potassium carbonate (0.54 g, 3.93 mmol) and 4-methoxy-α-toluenethiol (1.17 g, 10.48 mmol) in DMF (50 mL) under nitrogen. The mixture was refluxed for 5 h and allowed to cool to room temperature. The reaction mixture was then poured into H_2O and extracted with ethyl acetate. The organic layers were combined and washed with water. The organic layer was dried, filtered and concentrated under reduced pressure to give 3.76 g of crude material. The crude product was purified by column chromatography (ethyl acetate/hexane, 1:10) to give 4-[2-*tert*-butoxycarbonyl-3-(4-methoxy-benzylsulfanyl)-5-methyl-hexyl]-piperidine-1-carboxylic acid *tert*-butyl ester (1.77 g, 63 %).

(f) 3-Mercapto-5-methyl-2-piperidin-4-ylmethyl-hexanoic acid

A mixture of H_2O (2.6 mL) and TFA (26 mL) was frozen and then allowed to warm to room temperature under nitrogen. 4-[2-*tert*-butoxycarbonyl-3-(4-methoxy-benzylsulfanyl)-5-methyl-hexyl]-piperidine-1-carboxylic acid *tert*-butyl ester (2.62 g, 4.89 mmol) was added and the mixture was refluxed for 16 h. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The crude product was purified by reverse-phase column chromatography ($MeOH/H_2O$, 3:2) to give the title compound as the TFA salt (0.40 g, 22 %).

^1H NMR (300 MHz) (CD_3OD) δ 0.88 (d,), 0.94 (d,), 1.43 (m), 1.70 (br), 1.94 (m), 2.48 (m), 2.90 (m), 2.99 (br), 3.34 (m).

MS (+) 260.2 (M-TFA).

Example 4

3-Mercapto-4-phenyl-2-piperidin-4-ylmethyl-butyric acid

(a) 4-(2-*tert*-Butoxycarbonyl-4-phenyl-but-2-enyl)-piperidine-1-carboxylic acid *tert*-butyl ester

A solution of 4-[2-*tert*-butoxycarbonyl-2-(diethoxy-phosphoryl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (3.0 g) in DME (8 mL) was added dropwise to a suspension of sodium hydride (0.26 g, 10.51 mmol) in DME (8 mL) at 0^0C under nitrogen. The mixture was stirred for 0.75 h. Phenyl acetaldehyde (5.26 g, 43.81 mmol) was added dropwise to the mixture at 0^0C. The mixture was allowed to warm to room temperature and stirred for 16 h. The mixture was diluted with ether and washed with water. The organic layer was dried, filtered, and concentrated under reduced pressure to give 8.6 g of a yellow oil. The crude product was purified by column chromatography (ethyl acetate/hexane, 1:50 → 1:10) to give 4-(2-*tert*-butoxycarbonyl-4-phenyl-but-2-enyl)-piperidine-1-carboxylic acid *tert*-butyl ester (1.32 g, 62 % yield) for two reactions.

(b) 4-[2-*tert*-Butoxycarbonyl-3-(4-methoxy-benzylsulfanyl)-4-phenyl-butyl]-piperidine-1-carboxylic acid *tert*-butyl ester.

4-(2-*tert*-Butoxycarbonyl-4-phenyl-but-2-enyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.8 g, 1.93 mmol) in DMF (10 mL) was added to a suspension of potassium carbonate (0.20 g, 1.44 mmol)) and 4-methoxy-α-toluenethiol (0.54 mL, 3.85 mmol) in DMF (10 mL) under nitrogen. The mixture was heated to 75^0C for 24 h and allowed to cool to room temperature. The reaction mixture was then poured into H_2O and extracted with ethyl acetate. The organic layers were combined and washed with water. The organic layer was dried, filtered, and concentrated under reduced pressure to give 1.8 g crude material. The crude product was purified by column chromatography (ethyl acetate/ hexane, 1:10) to give 4-[2-*tert*-butoxycarbonyl-3-(4-methoxy-benzylsulfanyl)-4-phenyl-butyl]-piperidine-1-carboxylic acid *tert*-butyl ester (0.55 g, 50 %).

(c) 3-Mercapto-4-phenyl-2-piperidin-4-ylmethyl-butyric acid

A mixture of H_2O (0.65 mL) and TFA (6.5 mL) was frozen and then allowed to warm to room temperature under nitrogen. 4-[2-*tert*-butoxycarbonyl-3-(4-methoxy-benzylsulfanyl)-4-phenyl-butyl]-piperidine-1-carboxylic acid *tert*-butyl ester (0.65 g, 1.14 mmol) was added and the mixture was refluxed for 16 h. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The crude product was purified by

reverse-phase column chromatography ($MeOH/H_2O$, 1:1) to give the title compound as the TFA salt (0.27 g, 58 %).

^1H NMR (300 MHz) (DMSO) δ 1.22 (m), 1.49 (m), 1.67 (br), 2.21 (d,), 2.98 (m), 7.25 (m, 5H), 8.27 (br), 8.57 (br), 12.59 (br).

MS (+) 294.3 (M-TFA).

Example 5

2-(2-Amino-pyridin-4-ylmethyl)-3-mercapto-propionic acid

(a) *N*-(4-Methyl-pyridin-2-yl)-acetamide

2-Amino-4-methylpyridine (99.0 g, 91.5 mmol) in acetic anhydride (250 mL) was warmed to 70°C for two h. The mixture was cooled to room temperature and diethyl ether (100 mL) added. The product crystallized as white needle crystals. Filtering and drying *in vacuo* afforded *N*-(4-methyl-pyridin-2-yl)-acetamide (130 g, 95 %).

(b) 2-Acetylamino-isonicotinic acid

A mixture of *N*-(4-methyl-pyridin-2-yl)-acetamide(40.0 g, 0.26 mol) and water (400 mL) was heated at 90°C until the solution was homogeneous. $KMnO_4$ (100 g, 0.62 mol) was added carefully in small portions with vigorous mechanical stirring over 2 h. The reaction mixture was kept at 90-95°C for further 3 h before filtering through Celite while still hot. The filtrate was concentrated to about 100 mL and concentrated HCl was added to adjust the pH to about 4. The reaction flask was cooled in an ice bath and the white solid filtered off. The crystals were washed with cold water and chloroform and dried *in vacuo* giving 2-acetylamino-isonicotinic acid (12.0 g, 25 %).

(c) 2-Amino-isonicotinic acid ethyl ester

2-Acetylamino-isonicotinic acid (10.8 g, 60.0 mmol) was suspended in ethanol (150 mL) and $BF_3 \cdot OEt_2$ (22 mL, 138 mmol) was added. The mixture was refluxed overnight, and after cooling to room temperature 10 % $NaHCO_3$ (250 mL) was added. The product was extracted with chloroform and the combined organic extracts were washed with water and dried. Filtering and concentration afforded 2-amino-isonicotinic acid ethyl ester (7.46 g, 79 %) as pale yellow crystals.

(d) 2-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-isonicotinic acid ethyl ester

To a solution of 2-amino-isonicotinic acid ethyl ester (5.00 g, 30 mmol) in *t*-BuOH (45 mL) and acetone (15 mL) was added DMAP (50 mg, 0.41 mmol) and di-*t*-butyl dicarbonate (16.4 g, 75.0 mmol). The reaction was stirred at room temperature overnight and hexane (60 mL) was added. The reaction mixture was cooled in a refrigerator for 3 h and filtered to give 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-isonicotinic acid ethyl ester (8.71 g, 79 %).

(e) (4-Hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester

A solution of to 2-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-isonicotinic acid ethyl ester (35.0 g, 95.5 mmol) in THF (350 mL) was treated with $LiAlH_4$ (7.25 g, 191 mmol) and refluxed for 1 h under nitrogen. The reaction mixture was poured carefully onto crushed ice and the product extracted several times with $CHCl_3$ and $CHCl_3$: MeOH (9:1). The combined organic extracts were dried, filtered and concentrated under reduced pressure to give (4-hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (18.5 g, 86 %) as a pale yellow solid.

(f) (4-Bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester

(4-Hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (8.00 g, 35.6 mmol) was dissolved in CH_2Cl_2 (150 mL) and treated with PPh_3 (11.2 g, 42.8 mmol) under nitrogen. The reaction flask was cooled in an ice bath and CBr_4 (14.2 g, 42.8 mmol) was added in small portions. The ice bath was removed after 30 min and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and acetonitrile (50 mL) was added. The reaction flask was placed in a refrigerator for 3 h and the precipitate filtered and washed with cold acetonitrile. The white solid was dried *in vacuo* giving (4-bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (8.38 g, 82 %).

(g) 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid diethyl ester

To a solution of NaH (80%, 0.17 g, 4.00 mmol) in THF (5 mL) at 0°C under argon was added diethyl malonate (0.64 g, 4.00 mmol). After the mixture was stirred for 15 min the

mixture was added to a refluxed mixture of (4-bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (1.00 g, 3.48 mmol) in THF (10 mL), and the mixture was refluxed for 2 h. The mixture was concentrated under reduced pressure and the residue was partitioned between water and chloroform. The organic layer was washed with water and brine and dried. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (MeOH/ CH_2Cl_2, 1:100) to give 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid diethyl ester (0.80 g, 55 %).

(h) 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid monoethyl ester

A solution of KOH (0.19 g, 3.43 mmol) in ethanol (5 mL) was added to a solution of 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid diethyl ester (1.20 g, 3.27 mmol) in ethanol (5 mL) and methylene chloride (5 mL) at 0°C. The mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and water was added to the residue. The aqueous layer was washed with diethyl ether, acidified to pH 4 by 1M HCl, and extracted with methylene chloride. The organic layer was washed with water, brine and dried. After filtration and evaporation *in vacuo*, the crude product was purified by flash chromatography (CH_3OH/CH_2Cl_2, 1:20) to yield 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid monoethyl ester (0.90 g, 81 %).

(i) 2-(2-*tert*-Butoxycarbonylamino-pyridin-4-ylmethyl)-acrylic acid ethyl ester

A solution of diethylamine (0.26 g, 2.67 mmol) in methylene chloride (4 mL) was added to a mixture of 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-malonic acid monoethyl ester (0.90 g, 2.66 mmol) and 37 % aq. solution of formaldehyde (0.24 g, 3.00 mmol) at 0°C. The mixture was stirred for 5 h at room temperature and the mixture was poured onto ice-water and extracted with methylene chloride. The organic layer was washed with 5 % $NaHCO_3$ and dried. The crude product was purified by flash chromatography (1% methanol in CH_2Cl_2) to yield 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-acrylic acid ethyl ester (0.58 g, 71 %).

(j) 2-Acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonylamino-pyridin-4-yl)-propionic acid ethyl ester

A solution of 2-(2-*tert*-butoxycarbonylamino-pyridin-4-ylmethyl)-acrylic acid ethyl ester (0.48 g, 1.57 mmol) and triethylamine (0.17 g, 1.64 mmol) was added to thioacetic acid (3 mL) at 0°C under nitrogen. The mixture was stirred at room temperature for 4 h. The mixture was poured onto ice-water and extracted with CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$ (aq) and dried. The crude product was purified by flash chromatography (2.5 % MeOH in CH_2Cl_2) to give 2-acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonylamino-pyridin-4-yl)-propionic acid ethyl ester (0.60 g, 100 %).

(k) 2-Acetylsulfanylmethyl-3-(2-amino-pyridin-4-yl)-propionic acid ethyl ester

TFA (0.5 mL) was added to a solution of 2-acetylsulfanylmethyl-3-(2-*tert*-butoxycarbonylamino-pyridin-4-yl)-propionic acid ethyl ester (50 mg, 0.13 mmol) in methylene chloride under argon. The solution was stirred for 60 min and concentrated under reduced pressure to give crude 2-acetylsulfanylmethyl-3-(2-amino-pyridin-4-yl)-propionic acid ethyl ester (52 mg, 100 %).

^{1}H NMR (500 MHz, CD_3OD_3): δ 1.15 (t, 3H), 2.32 (s, 3H), 2.73-2.83 (m, 2H), 2.86-2.93 (m, 1H), 3.01-3.07 (dd, 1H), 3.12-3.18 (dd, 1H), 4.03-4.12 (m, 2H), 6.39 (s, 1H), 6.43 (d, 1H), 7.77 (d, 1H).

(l) 2-(2-Amino-pyridin-4-ylmethyl)-3-mercapto-propionic acid

2-Acetylsulfanylmethyl-3-(2-amino-pyridin-4-yl)-propionic acid ethyl ester (52 mg, 0.13 mmol) was dissolved in conc. HCl (2 mL) under argon. The solution was heated to reflux for 1 h. Concentration under reduced pressure gave the title compound as the hydrochloride salt (32 mg, 100 %).

^{1}H NMR (500 MHz. CD_3OD): δ 2.70 (bs, 2H), 2.85-3.0 (m, 3H), 6.76 (bs, 1H), 6.81 (bs, 1H), 7.67 (bs, 1H).

MS (+) 213 (M+1).

Example 6

3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-propionic acid

(a) 6-Amino-nicotinic acid ethyl ester

2-Amino-5-pyridinecarboxylic acid (25.0 g, 181 mmol) was suspended in ethanol (190 mL) and $SOCl_2$ (15 mL, 206 mmol) was added. The mixture was refluxed for 10 hs and more $SOCl_2$ (16 mL) was added. After 3 days with reflux (and more $SOCl_2$ (10 mL) added each day), the reaction mixture was cooled to room temperature and diethyl ether was added. After 24 h at – 20°C the mixture was filtered. The crude salt was dissolved in methanol (214 mL) and a solution of NaOH (40.0 g, 23.5 mmol) in methanol (90 mL) was added. The reaction mixture was stirred for 1 h and THF (270 mL) was added. The reaction mixture was filtered through a plug of silica (THF/MeOH) and concentrated under reduced pressure to give 6-amino-nicotinic acid ethyl ester (36.2 g, 97 %).

(b) 6-*tert*-Butoxycarbonylamino-nicotinic acid ethyl ester

To a solution of 6-amino-nicotinic acid ethyl ester (36.0 g, 217.0 mmol) in *t*-BuOH (308 mL) and acetone (103 mL) was added DMAP (0.53 g, 4.34 mmol) and di-*t*-butyl dicarbonate (72.0 g, 330 mmol). The reaction was stirred at room temperature for 10 h followed by addition of more di-*t*-butyl dicarbonate (2.60 g). After 10 h stirring at room temperature hexane (470 mL) was added. The reaction mixture was cooled to -20°C for 2 h and filtered. The filtrate was washed with hexane/dichloromethane (3:1) and and dried *in vacuo* to give 6-*tert*-butoxycarbonylamino-nicotinic acid ethyl ester (40.5 g, 70 %).

(c) (5-Hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester

To a stirred solution of 6-*tert*-butoxycarbonylamino-nicotinic acid ethyl ester (3.50 g, 13.1 mmol) in THF (20 mL) under nitrogen was added $LiAlH_4$ (0.91 g, 24.0 mmol) in THF (20 mL) over a period of 2 h. The reaction mixture was stirred for 6 h, then NH_4Cl (sat.) was added (carefully) until neutral solution. The mixture was filtered and concentrated under reduced pressure to give (5-hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (2.00 g, 68 %).

(d) (5-Bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester

Triphenylphosphine (8.70 g, 33.1 mmol) and carbontetrabromide (17.0 g, 51.2 mmol) were added to a suspension of (5-hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (7.00 g, 31.2 mmol) in CH_2Cl_2 (200 mL) at room temperature. Stirring was continued for 5

h followed by evaporation of the solvent. Acetonitrile (200 mL) was added and the mixture was cooled to -20°C for 2 h. The mixture was then filtered and the crystalline residue washed with cold acetonitrile (2 x 10 mL), to give (5-bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (5.96 g, 67%).

(e) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid diethyl ester

To a suspension of NaH (0.49 g, 16.3 mmol, 80%) in THF (15 mL) at 0°C was added diethyl malonate (2.61 g, 16.3 mmol). The mixture was stirred for 15 min and was then added dropwise to a refluxed mixture of (5-bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (3.90 g, 13.6 mmol) in THF (25 mL), and the resulting solution was refluxed for 15 min. After evaporation of the solvent, the crude product was purified by flash chromatography (methanol/CH_2Cl_2, 1:100 → 2.5:100) to give 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid diethyl ester (2.18 g, 44 %).

(f) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid monoethyl ester

A solution of KOH (0.37 g, 6.54 mmol) in ethanol (5 mL) was added to a solution of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid diethyl ester (2.18 g, 5.95 mmol) in ethanol (25 mL) and methylene chloride (10 mL) at 0°C. The mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and the residue dissolved in water. The aqueous layer was washed with ether, acidified to pH 4 by 1M HCl and extracted with methylene chloride. The organic layer was washed with water, brine and dried. After filtration and concentration under reduced pressure, the crude product was purified by flash chromatography (methanol/ CH_2Cl_2, 1:20) to yield 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.00 g, 50 %).

(g) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-acrylic acid ethyl ester

Diethylamine (0.29 g, 3.00 mmol), water (2 mL) and methylene chloride (2 mL) was added to a mixture of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.00 g, 2.96 mmol) and 37 % aq. solution of formaldehyde (0.25 g, 3.05 mmol) at 0°C. The mixture was stirred for 16 h at room temperature and then poured onto ice-water and extracted with methylene chloride. The organic layer was washed with 5 %

$NaHCO_3$ and dried. Filtration and concentration under reduced pressure gave 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.75 g, 83 %).

(h) 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-propionic acid ethyl ester

2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.49 g, 1.60 mmol) and triethylamine (0.17 g, 1.64 mmol) were added to thioacetic acid (3 mL) at 0°C. The mixture was stirred at room temperature for 6 h. The mixture was poured onto ice-water and extracted with CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$ and dried. The crude product was purified by flash chromatography ($MeOH/CH_2Cl_2$, 2.5:100) to give 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-propionic acid ethyl ester (0.36 g, 61 %).

(i) 2-Acetylsulfanylmethyl-3-(6-amino-pyridin-3-yl)-propionic acid ethyl ester

TFA (0.5 mL) was added to a solution of 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-propionic acid ethyl ester (100 mg, 0.26 mmol) in methylene chloride (2 mL) under argon. The solution was stirred for 60 min and concentrated under reduced pressure to give crude 2-acetylsulfanylmethyl-3-(6-amino-pyridin-3-yl)-propionic acid ethyl ester (104 mg, 100 %).

^1H NMR (500 MHz, CD_3OD): δ 1.21 (t, 3H), 2.33 (s, 3H), 2.78-2.97 (m, 3H), 3.05-3.13 (m, 1H), 3.14-3.21 (m, 1H), 4.08-4.15 (m, 2H), 6.99 (d, 1H), 7.69 (s, 1H), 7.85 (d, 1H).

(j) 3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-propionic acid

2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-propionic acid ethyl ester (38 mg, 0.096 mmol) was dissolved in conc. HCl (2.0 mL) under argon. The solution was stirred at room temperature for 1 h and then heated to reflux for 1 h. Concentration under reduced pressure gave the title compound (25.7 mg, 100 %) as the hydrochloride salt.

^1H NMR (500 MHz, CD_3OD): δ 2.74-2.78 (m, 2H), 2.84-2.94 (m, 3H), 7.02 (d, 1H), 7.72 (s, 1H), 7.89 (d, 1H).

MS (+) 213 (M+1).

Example 7

3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid

(a) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid *tert*-butyl ester ethyl ester

A solution of tert-butyl ethyl methylmalonate (457 mg, 2.26 mmol) in DMF (4 mL) was added dropwise to a suspension of NaH (90 mg, 2.26 mmol, 60 % in oil) in DMF (4 mL). The reaction mixture was stirred for 20 min. A solution of (5-bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (500 mg, 1.74 mmol) in DMF (2.5 mL) was added and the reaction was stirred for 70 min. EtOAc was added and the mixture was washed with water and brine, dried and concentrated under reduced pressure. Chromathography (Heptane/ EtOAc, 3:1 → 1:3) gave 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid *tert*-butyl ester ethyl ester (437 mg, 61 % yield).

(b) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid mono-*tert*-butyl ester

1M NaOH (2 mL) was added to a solution of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid *tert*-butyl ester ethyl ester (0.42g, 1.03 mmol) in THF/EtOH (4 mL, 1:1) . The reaction mixture was stirred at 50°C for 16 h. CH_2Cl_2 was added and the mixture was washed with 0.5 M HCl and brine and dried. Concentration under reduced pressure gave 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid mono-*tert*-butyl ester (348 mg, 89 %).

(c) 3-(6-*tert*-Butoxycarbonylamino-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid *tert*-butyl ester

Methyl chloroformate (75 µL, 0.92 mmol) was added dropwise to a solution of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-methyl-malonic acid mono-*tert*-butyl ester (348 mg, 0.915 mmol) and Et_3N (123 µL, 0.92 mmol) in THF (6 mL). The reaction mixture was stirred for 20 min., filtered and added dropwise to a suspension of $NaBH_4$ (39 mg, 1.04 mmol) in THF (6 mL) at 0°C. The reaction was stirred for 16 h at room temperature. 0.2 M HCl was added followed by EtOAc. The organic phase was washed with brine and dried. Concentration under reduced pressure followed by chromathography

(toluene/EtOAc, 3:1 → 1:3) gave 3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid *tert*-butyl ester (190 mg, 57 %).

(d) 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-methyl-propionic acid *tert*-butyl ester

Diethyl azodicarboxylate (160 µL, 1.01 mmol) was added dropwise to a solution of 3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid *tert*-butyl ester (180 mg, 0.49 mmol) and triphenylphosphine (266 mg, 1.01 mmol) in THF (6 mL) and the reaction was stirred for 5 min. Thiolacetic acid (96 µL, 1.34 mmol) was added and the reaction was stirred for 16 h. Concentration under reduced pressure followed by chromathography (toluene/EtOAc, 10:1 → 1:1) gave 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-methyl-propionic acid *tert*-butyl ester (137 mg, 65 %).

(e) 3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid

2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-2-methyl-propionic acid *tert*-butyl ester (4 mg, 9.4 µmol) was dissolved in conc. HCl under argon. The solution was heated to reflux for 1 h. Concentration under reduced pressure yielded the title compound as the hydrochloride salt (2.5 mg, 100 %).

^{1}H NMR (500 MHz, CD_3OD): δ 1.20 (s, 3H), 2.62 (d, 1H), 2.76-2.83 (m, 2H), 2.95 (d, 1H), 6.94 (d, 1H), 7.64 (d, 1H), 7.80 (dd, 1H).

MS (+) 227 (M+1).

Example 8

2-(6-Amino-pyridin-3-ylmethyl)-2-mercaptomethyl-butyric acid

(a) [5-(5-Ethyl-2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin-2-yl]-carbamic acid *tert*-butyl ester

(5-Bromomethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (1.0 g, 3.48 mmol) was added to a solution of 2,2-dimethyl-5-ethyl-1,3-dioxane-4,6-dione (600 mg, 3.48 mmol) and triethylamine (0.51 mL, 3.66 mmol) in dimethyl sulfoxide (40 mL) under nitrogen. The reaction mixture was stirred over night and water (100 mL) was added. Filtration of the

precipitate gave [5-(5-ethyl-2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin-2-yl]-carbamic acid *tert*-butyl ester (1.15 g, 87 %).

(b) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethyl-malonic acid monoethyl ester

A solution of sodium metal (140 mg, 6.08 mmol) in ethanol (20 mL) was added to a solution of [5-(5-ethyl-2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin-2-yl]-carbamic acid *tert*-butyl ester (1.15 g, 3.04 mmol) in ethanol (10 mL). The reaction was stirred for 90 min. and methylene chloride was then added. The mixture was washed with 0.5 M HCl, dried and concentrated under reduced pressure to give 2-(6-*tert*-butoxy-carbonylamino-pyridin-3-ylmethyl)-2-ethyl-malonic acid monoethyl ester (1.05 g, 95%).

(c) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethyl)-2-hydroxymethyl-butyric acid ethyl ester

Methyl chloroformate (150 µL, 1.95 mmol) was added dropwise to a solution of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-ethyl-malonic acid monoethyl ester (700 mg, 1.91 mmol) and Et_3N (275 µL, 1.97 mmol) in THF (15 mL) at -20°C under nitrogen. The reaction mixture was stirred for 50 min., filtered and added dropwise to a suspension of $NaBH_4$ (80 mg, 2.1 mmol) in THF (15 mL) at -20°C. The reaction was stirred for 16 h at room temperature. 0.2 M HCl was added followed by methylene chloride. The organic phase was washed with brine and dried. Concentration under reduced pressure followed by chromathography (toluene/EtOAc, 3:1 → 1:3) gave 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-hydroxymethyl-butyric acid ethyl ester (300 mg, 45 %).

(d) 2-Acetylsulfanylmethyl-2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-butyric acid ethyl ester

Diisopropyl azodicarboxylate (296 µL, 1.53 mmol) was added dropwise to a solution of triphenylphosphine (402 mg, 1.53 mmol) in THF (4 mL) at 0°C under argon and the reaction was stirred for 30 min. A solution of thiolacetic acid (109 µL, 1.53 mmol) and 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-2-hydroxymethyl-butyric acid ethyl ester (0.27 g, 0.77 mmol) in THF (2 mL) was added dropwise during 10 min. The reaction was stirred for 60 min. at 0°C and then for 16 h at room temperature. Concentration under

reduced pressure followed by chromathography (heptane/EtOAc, 10:1 → 1:1) gave 2-acetylsulfanylmethyl-2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-butyric acid ethyl ester (193 mg, 61 %).

(e) 2-(6-Amino-pyridin-3-ylmethyl)-2-mercaptomethyl-butyric acid

2-Acetylsulfanylmethyl-2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethyl)-butyric acid ethyl ester (12.3 mg, 30 μmol) was dissolved in conc. HCl (2 mL) under argon. The solution was heated to reflux for 24 h. Concentration under reduced pressure gave the title compound as the hydrochloride salt (8.3 mg, 100 %).

^{1}H NMR (500 MHz, CD_3OD): δ 0.91 (t, 3H), 1.71 (m, 2H), 2.68 (m, 2H), 2.92 (m, 2H), 6.96 (d, 1H), 7.65 (bs, 1H), 7.82 (dd, 1H).

MS (-) 239 (M-1).

Example 9

3-(6-Amino-5-methyl-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid

(a) 2-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-3-Methyl-5-(2,2,5-trimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin

2-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-bromomethyl-3-methyl-pyridin (1.6 g, 4.0 mmol) was added to a solution of 2,2,5-trimethyl-1,3-dioxane-4,6-dione (630 mg, 4.0 mmol) and triethylamine (0.58 mL, 4.2 mmol) in dimethyl sulfoxide (40 mL). The reaction mixture was stirred overnight and water (100 mL) was added. The mixture was extracted with EtOAc, the combined organic phases washed with water and brine and dried. Concentration under reduced pressure gave crude 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-3-methyl-5-(2,2,5-trimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin (2.06 g)

(b) 2-(6-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-2-methyl-malonic acid monoethyl ester

A solution of sodium metal (184 mg, 8.0 mmol) in ethanol (20 mL) was added to a solution of crude 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-3-methyl-5-(2,2,5-trimethyl-4,6-dioxo-[1,3]dioxan-5-ylmethyl)-pyridin (2.06 g, ~4.0 mmol) in ethanol (20 mL) under argon. The reaction was stirred for 60 min. and methylene chloride was then added. The

mixture was washed with 0.5 M HCl and brine, dried and concented under reduced pressure to give crude 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-2-methyl-malonic acid monoethyl ester (1.9 g)

(c) 3-(6-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid ethyl ester

Methyl chloroformate (338 µL, 4.4 mmol) was added dropwise to a solution of crude 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-2-methyl-malonic acid monoethyl ester (1.9 g) and Et_3N (641 µL, 4.6 mmol) in THF (30 mL) at -20°C. The reaction mixture was stirred for 50 min., filtered and added dropwise to a suspension of $NaBH_4$ (182 mg, 4.8 mmol) in THF (30 mL) at -20°C. The reaction was stirred for 16 h at room temperature. 0.5 M HCl was added followed by methylene chloride. The organic phase was washed with brine and dried. Concentration under reduced pressure followed by chromathography (toluene/EtOAc, 10:1 → 1:3) gave 3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)-amino]-5-methyl-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid ethyl ester (885 mg, 49 %).

(d) 2-Acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester

Diisopropyl azodicarboxylate (755 µL, 3.91 mmol) was added dropwise to a solution of triphenylphosphine (1.026 g, 3.91 mmol) in THF (10 mL) at 0°C and the reaction was stirred for 30 min. A solution of thiolacetic acid (279 µL, 3.91 mmol) and 3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-2-hydroxymethyl-2-methyl-propionic acid ethyl ester (885 mg, 1.96 mmol) in THF (5 mL) was added dropwise during 10 min. The reaction was stirred for 60 min. at 0°C and then for 16 h at room temperature. Concentration under reduced pressure followed by chromathography (heptane/EtOAc, 10:1 → 1:3) gave impure 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester (1.46 g)

(e) 2-Acetylsulfanylmethyl-3-(6-amino-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester

Crude 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester (1.46g) was dissolved in TFA (5 mL) and stirred for 60 min. Concentration under reduced pressure followed by chromathography (toluene/EtOAc, 1:1 → 1:10 → 0:1) gave slightly impure 2-acetylsulfanylmethyl-3-(6-amino-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester (696 mg, 84%)

(f) 3-(6-Amino-5-methyl-pyridin-3-yl)-2-mercaptomethyl-2-methyl-propionic acid

2-Acetylsulfanylmethyl-3-(6-amino-5-methyl-pyridin-3-yl)-2-methyl-propionic acid ethyl ester (17 mg, 40 µmol) was dissolved in conc. HCl (2 mL) under argon. The solution was heated to reflux for 150 min. Concentration under reduced pressure gave the title compound as the hydrochloride salt (10.7 mg, 96 %).

^{1}H NMR (500 MHz, CD_3OD): δ 1.20 (s, 3H), 2.23 (s, 3H), 2.61 (d, 1H), 2.79 (2d, 2H), 2.94 (d, 1H), 7.55 (m, 1H), 7.69 (m, 1H).

MS (+) 241 (M+1).

Example 10

3-Mercapto-2-[(piperidine-4-carbonyl)-amino]-propionic acid

CH_2Cl_2 (70 mL) was added to 4-methoxytrityl chloride resin (7 g, L=0.91 mmol/g, 6.37 mmol) under argon and the resin was allowed to swell for 10 min and 2-amino-3-mercapto-propionic acid ethyl ester HCl-salt (5.9 g, 32 mmol) was added. TFA (70 mL) was then added in small portions over 10 min. The slurry was shaken at room temperature for 1 h and concentrated under reduced pressure. When almost dry, toluene (150 mL) was added and the mixture was again concentrated under reduced pressure. This procedure was repeated twice. The now yellow resin was washed with DMF (3x60 mL), CH_2Cl_2 (2x60 mL), TEA:CH_2Cl_2 (1:1, 2x60 mL), CH_2Cl_2 (2x60 mL), MeOH (2x60 mL) and dried under vacuum overnight.

To calculate the loading of 2-amino-3-mercapto-propionic acid ethyl ester on the resin, 50 mg of the product was treated with 10% TFA in CH_2Cl_2 for 1 minute, and this procedure was repeated 4 times. The mixture was concentrated under reduced pressure to give 2-

Amino-3-mercapto-propionic acid ethyl ester (9.8 mg), indicating a loading of about 0.6 mmol/g.

A solution of piperidine-1,4-dicarboxylic acid mono-*tert*-butyl ester (28 mg, 0.12 mmol) in DMF (1 mL) was added to the resin (100 mg, L=0.6 mmol/g, 0.06 mmol) in a plastic syringe, followed by PyBOP (62 mg, 0.12 mmol) in DMF (0.5 mL) and DIPEA (41 µL, 0.24 mmol). The reaction was left at room temperature for 2 h with occasional stirring and the procedure was repeated once more. The resin was then washed with DMF (2x2 mL), CH_2Cl_2 (2x2 mL), MeOH (2x2 mL), CH_2Cl_2 (2x2 mL) and THF (2x2 mL).
THF (800 µL) was added to the syringe and the resin was allowed to swell for 10 min. Then water (250 µL) and 10 M NaOH (50 µL) were added. The reaction was left at room temperature for 16 h with occasional stirring. The resin was then washed with THF:water (1:1, 2x2 mL), THF (2x2 mL), CH_2Cl_2 (2x2 mL), MeOH (2x2 mL) and CH_2Cl_2 (2x2 mL).

10 % TFA in CH_2Cl_2 (1 mL) was added to the syringe and after 5 min the solution was collected in a tared vial. This procedure was repeated one more time and the combined organic phases were concentrated under reduced pressure to yield the title compound as the TFA salt (15.3 mg, 74 %).
^{1}H NMR (500 MHz, CD_3OD): δ 1.85-2.10 (m, 4H), 2.65-2.72 (m, 1H), 2.85-2.92 (m, 1H), 2.95-3.08 (m, 3H), 3.40-3.47 (m, 2H), 4.55-4.60 (m, 1H).

Example 11

2-[(Azetidine-2-carbonyl)-amino]-3-mercapto-propionic acid

The title compound was prepared from azetidine-1,2-dicarboxylic acid 1-*tert*-butyl ester by the method described in Example 14. Yield: 13.8 mg (72 %).
^{1}H NMR (500 MHz, CD_3OD): δ 2.54-2.63 (m, 1H), 2.82-3.05 (m, 3H), 3.93-4.15 (m, 2H), 4.66-4.71 (m, 1H), 5.05-5.10 (m, 1H).

Example 12

3-Mercapto-2-[(piperidine-3-carbonyl)-amino]-propionic acid

The title compound was prepared from piperidine-1,3-dicarboxylic acid 1-*tert*-butyl ester by the method described in Example 14. Yield: 15.1 mg (73 %).

^{1}H NMR (500 MHz, CD_3OD): δ 1.73-2.10 (m, 4H), 2.84-2.92 (m, 2H), 2.95-3.14 (m, 2H), 3.15-3.29 (m, 3H), 4.56-4.62 (m, 1H).

Example 13

2-[(Azetidine-3-carbonyl)-amino]-3-mercapto-propionic acid

The title compound was prepared from azetidine-1,3-dicarboxylic acid mono-*tert*-butyl ester by the method described in Example 14. Yield: 13.5 mg (71 %).

^{1}H NMR (500 MHz, CD_3OD): δ 2.86-3.02 (m, 2H), 3.72-3.80 (m, 1H), 4.20-4.24 (d, 4H), 4.62-4.67 (m, 1H).

Example 14

3-(6-Amino-5-methyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid

(a) 5-Bromo-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-3-methyl-pyridin

2-Amino-5-bromo-3-methylpyridine (15.0 g, 80.2 mmol) in tert-butanol was treated with di-tert-butyl dicarbonate (43.6 g, 200 mmol) and DMAP (0.60 g, 4.91 mmol). The reaction mixture was left at ambient temperature overnight and was then concentrated under reduced pressure. Hexane was added and the product precipitated as a solid. Filtering afforded 5-bromo-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-3-methyl-pyridin (22.0 g, 71 %).

(b) 2-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-(*tert*-butyl-dimethyl-silanyloxymethyl)-3-methylpyridin

A solution of 5-bromo-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-3-methyl-pyridin (26.0 g, 67.1 mmol), *tert*-butyl-dimethyl-tributylstannanylmethoxy-silane (47.6 g, 109 mmol), and bis(triphenylphosphine)palladium(II) dichloride (0.90 g, 1.42 mmol) in 1,2-dichloroethane (80 mL) was stirred at 90°C for two days. The mixture was cooled to 0°C and diethyl ether (200 mL) was added followed by saturated aqueous potassium fluoride (40 mL). The mixture was stirred vigourously for 30 min and filtered. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 100:0 → 95:5) gave 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-(*tert*-butyl-dimethyl-silanyloxymethyl)-3-methylpyridin (18.0 g, 59 %).

(c) 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-hydroxymethyl-3-methylpyridin

Tetrabutylammonium fluoride (25.1 g, 79.6 mmol) was added to a solution of 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-(*tert*-butyl-dimethyl-silanyloxymethyl)-3-methylpyridin (18.0 g, 39.8 mmol) in THF (150 mL). The reaction mixture was stirred overnight at room temperature. Concentration under reduced pressure followed by flash chromatography (hexane/EtOAc, 50:50) gave 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-hydroxymethyl-3-methylpyridin (8.0 g, 59 %).

(d) 5-Bromomethyl-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]- 3-methylpyridin

Triphenylphosphine (7.43 g, 28.3 mmol) and CBr_4 (9.49 g, 28.6 mmol) was added to a solution of 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-hydroxymethyl-3-methylpyridin (8.00 g, 23.6 mmol) in dichloromethane (220 mL) at 0°C. The reaction mixture was stirred for 3 h and was then concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 80:20) gave 5-bromomethyl-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-3-methylpyridin (8.0 g, 77 %).

(e) 2-(6-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)- malonic acid diethyl ester

To a suspension of NaH (0.24 g, 6.0 mmol, 60%) in DMF (5 mL) was added diethyl malonate (0.91 mL, 6.0 mmol) and the mixture was stirred for 15 min. A solution 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-bromomethyl-3-methyl-pyridin (2.0 g, 5.0 mmol) in DMF (5 mL) was added and the resulting solution stirred for 120 min at 60°C. Ethyl acetate was added and the mixture was washed with water and brine and dried. After evaporation of the solvent, the crude product was purified by flash chromatography (CH_3OH/CH_2Cl_2, 1:100 → 1:20) to give 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (1.2 g, 50 %).

(f) 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester

A solution of KOH (154 mg, 2.75 mmol) in ethanol (2 mL) was added to a solution 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (1.2 g, 2.50 mmol) in ethanol (10 mL) and methylene chloride (4 mL) at 0°C. The

mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and the residue dissolved in water. Ethyl acetate was added and the organic layer was washed with 0.5 M HCl, water, brine and dried. After filtration and concentration under reduced pressure gave crude 2-(6-[*N,N*-bis(*tert*-butoxy-carbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.0 g, 88%).

(g) 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester

Diethylamine (0.26 g, 2.67 mmol) was added a mixture of 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.0 g, 2.2 mmol) and 37 % aq. solution of formaldehyde (0.24 g, 3.00 mmol) in methylene chloride (2 mL) at 0 °C. The mixture was stirred for 16 h at room temperature and ethyl acetate was added. The organic layer was washed with water and 5 % $NaHCO_3$ and dried. Concentration under reduced pressure followed by flash chromatography (toluene/ethyl acetate, 3:1 → 1:2) gave 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.68 g, 73 %).

(h) 2-Acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester

Triethylamine (0.234 mL, 1.68 mmol) was added to a solution of 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.68 g, 1.61 mmol) in thioacetic acid (3 mL) at 0°C. The mixture was stirred at room temperature for 16 h. Ethyl acetate was added and the organic phase was washed with water, saturated $NaHCO_3$ and brine and dried. The crude product was purified by flash chromatography (toluene/ethyl acetate, 3:1 → 1:2) to give pure 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (489 mg, 61%) and slightly impure 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (0.34 g, 43%).

(i) 3-(6-Amino-5-methyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid

2-Acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (17 mg, 0.034 mmol) was dissolved in conc. HCl (3.0 mL). The

solution was heated to reflux for 1 h. Concentration under reduced pressure gave the title compound (8.9 mg, 100 %) as the hydrochloride salt.

^{1}H NMR (500 MHz, CD_3OD): δ 2.26 (s, 3H), 2.72-2.75 (m, 2H), 2.83-2.91 (m, 3H), 7.60 (s, 1H), 7.77 (s, 1H).

MS (+) 227 (M+1).

Example 15

3-(6-Amino-4-methyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid

(a) 2-Amino-5-bromo-4-methylpyridine

2-Amino-4-methylpyridine (110 g, 1.02 mol) in hydrobromic acid (1 L, 48%) was stirred at 70°C and a solution of hydrogen peroxide (300 mL, 15 %) was added dropwise over a one h at such a rate that the temperature of the reaction mixture remained at 70 - 80°C. The mixture was stirred for 90 min at 70°C and poured onto crushed ice. The pH was adjusted to 4-5 with sodium carbonate and the precipitated solid (containing mostly dibrominated products) was filtered off and discarded. The pH was subsequently raised to 9 and the precipitated product collected by filtration. Recrystallization from toluene gave 2-Amino-5-bromo-4-methylpyridine (76.3 g, 40 %).

(b) 2-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-bromo-4-methylpyridin

2-Amino-5-bromo-4-methylpyridine (5.70 g, 30.5 mmol) in chloroform was treated with di-tert-butyl dicarbonate (20.0 g, 91.60 mmol) and DMAP (0.60 g, 4.91 mmol). The reaction mixture was left at ambient temperature overnight and was then concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 95:5) gave 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-bromo-4-methylpyridin (8.02 g, 68 %).

(c) 2-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-5-(*tert*-butyl-dimethyl-silanyloxymethyl)-4-methylpyridin

A solution of 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-bromo-4-methylpyridin (15.0 g, 38.70 mmol), *tert*-butyl-dimethyl-tributylstannanylmethoxy-silane (25.4 g, 58.3 mmol), and bis(triphenylphosphine)palladium(II) dichloride (0.90 g, 1.42 mmol) in 1,2-dichloro-ethane (50 mL) was stirred at 90°C for two days. The mixture was cooled to 0°C and

diethyl ether (200 mL) was added followed by saturated aqueous potassium fluoride (40 mL). The mixture was stirred vigourously for 30 min and filtered. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 95:5) gave 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-(*tert*-butyl-dimethyl-silanyloxymethyl)-4-methylpyridin (10.0 g, 57 %).

(d) 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-hydroxymethyl-4-methylpyridin

Tetrabutylammonium fluoride (13.9 g, 44.1 mmol) was added to a solution of 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-(*tert*-butyl-dimethyl-silanyloxymethyl)-4-methylpyridin (10.0 g, 24.3 mmol) in THF (100 mL). The reaction mixture was stirred for 3 h at room temperature. Concentration under reduced pressure followed by flash chromatography (hexane/EtOAc, 50:50) gave 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-hydroxymethyl-4-methylpyridin (5.0 g, 67 %).

(e) 5-Bromomethyl-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]- 4-methylpyridin

Triphenylphosphine (4.69 g, 17.9 mmol) and CBr_4 (4.89 g, 14.8 mmol) was added to a solution of 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-hydroxymethyl-4-methylpyridin (5.00 g, 22.0 mmol) in dichloromethane (130 mL) at 0°C. The reaction mixture was stirred for 3 h and was then diluted with dichloromethane. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 80:20) gave 5-bromomethyl-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methylpyridin (5.35 g, 90 %).

(f) 2-(6-[*N,N*-bis(*tert*-Butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)- malonic acid diethyl ester

To a suspension of NaH (0.24 g, 6.0 mmol, 60%) in DMF (5 mL) was added diethyl malonate (0.91 mL, 6.0 mmol) and the mixture was stirred for 15 min. A solution 2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-bromomethyl-4-methyl-pyridin (2.0 g, 5.0 mmol) in DMF (5 mL) was added and the resulting solution stirred for 120 min at 60 °C. Ethyl acetate was added and the mixture was washed with water and brine and dried. After evaporation of the solvent, the crude product was purified by flash chromatography (CH_3OH/CH_2Cl_2, 1:100 →1:20) to give a pure fraction 2-(6-[*N,N*-bis(*tert*-butoxy-

carbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (1.15 g, 48 %) and an impure fraction 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (1.1 g).

(g) 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester

A solution of KOH (141 mg, 2.52 mmol) in ethanol (2 mL) was added to a solution 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester (1.1 g, 2.29 mmol) in ethanol (10 mL) and methylene chloride (4 mL) at 0°C. The mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure and the residue dissolved in water. Ethyl acetate was added and the organic layer was washed with 0.5 M HCl, water, brine and dried. After filtration and concentration under reduced pressure gave crude 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)-amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.0 g, 97 %).

(h) 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester

Diethylamine (0.26 g, 2.67 mmol) was added a mixture of 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-malonic acid monoethyl ester (1.0 g, 2.2 mmol) and 37 % aq. solution of formaldehyde (0.24 g, 3.00 mmol) in methylene chloride (2 mL) at 0°C. The mixture was stirred for 16 h at room temperature and ethyl acetate was added. The organic layer was washed with water and 5 % $NaHCO_3$ and dried. Concentration under reduced pressure followed by flash chromatography (toluene/ethyl acetate, 3:1 → 1:1) gave 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.81 g, 88 %).

(i) 2-Acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-yl)-propionic acid ethyl ester

Triethylamine (0.279 mL, 2.0 mmol) was added to a solution of 2-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-ylmethyl)-acrylic acid ethyl ester (0.8 g, 1.9 mmol) in thioacetic acid (3 mL) at 0°C. The mixture was stirred at room temperature for 16 h. Ethyl acetate was added and the organic phase was washed with water, saturated

$NaHCO_3$ and brine and dried. The crude product was purified by flash chromatography (toluene/ethyl acetate, 3:1 → 1:2) to give pure 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-5-methyl-pyridin-3-yl)-propionic acid ethyl ester (200 mg, 21 %) and slightly impure 2-acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-yl)-propionic acid ethyl ester (0.68 g).

(j) 3-(6-Amino-4-methyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid

2-Acetylsulfanylmethyl-3-(6-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-4-methyl-pyridin-3-yl)-propionic acid ethyl ester (36 mg, 0.072 mmol) was dissolved in conc. HCl (3.0 mL). The solution was heated to reflux for 1 h. Concentration under reduced pressure gave the title compound (18.7 mg, 98 %) as the hydrochloride salt.

^{1}H NMR (500 MHz, CD_3OD): δ 2.42 (s, 3H), 2.72-2.95 (m, 5H), 6.81 (s, 1H), 7.58 (s, 1H).

MS (+) 227 (M+1).

Example 16

2-Mercaptomethyl-3-piperidin-4-yl-butyric acid

(a) 4-formyl-piperidine-1-carboxylic acid *tert*-butyl ester

Periodinane (26.9 g, 63.5 mmol) was added to a solution of 1-*tert*-butoxycarbonyl-piperidine-4-methanol (10.5 g, 48.8 mmol) in methylene chloride (200 mL) and the mixture was stirred for 90 min. Diethyl ether was added and precipitates were removed by extraction with 10 % $Na_2S_2O_3$/saturated $NaHCO_3$ (1:1, 300 mL). The organic layer was washed with 0.5 M NaOH and brine, dried and concentrated under reduced pressure. Flash chromatography (hexane/EtOAc, 8:2) gave 4-formyl-piperidine-1-carboxylic acid *tert*-butyl ester (8.5 g, 81 %).

(b) 2-(1-*tert*-Butoxycarbonyl-piperidin-4-ylmethylene)-malonic acid diethyl ester

To a solution of diethyl malonate (710 µL, 4.7 mmol) and 4-formyl-piperidine-1-carboxylic acid *tert*-butyl ester (1.0 g, 4.7 mmol) in methylene chloride (5 mL) was added piperidine (46 µL, 0.47 mmol) and acetic acid (27 µL, 0.47 mmol). The reaction mixture was stirred for 72 h at room temperature and then for 16 h at 45°C. EtOAc was added and the mixture was washed with water and brine, dried and concentrated under reduced

pressure. The crude was purified by flash chromatography (heptane/EtOAc, 3:1 → 1:3) to give 2-(1-*tert*-butoxycarbonyl-piperidin-4-ylmethylene)-malonic acid diethyl ester (0.69 g, 40 %).

(c) 2-[1-(1-*tert*-Butoxycarbonyl-piperidin-4-yl)-ethyl]-malonic acid diethyl ester
MeLi (5.34 mL, 8.54 mmol, 1.6 M in diethyl ether) was added dropwise to a slurry of CuI (0.74 g, 3.88 mmol) in THF (5 mL) at -78°C under argon and the mixture was stirred for 30 min. A solution of 2-(1-*tert*-butoxycarbonyl-piperidin-4-ylmethylene)-malonic acid diethyl ester (0.69 g, 1.94 mmol) in THF (5 mL) was added dropwise and the reacton mixture was stirred for 120 min at -78°C and was then allowed to warm to room temperature during 60 min. Concentrated aqueous NH_4OH was added and the mixture was then extracted with EtOAc, washed with concentrated aqueous NH_4OH and brine, dried and concentrated under reduced pressure. The crude was purified by flash chromatography (heptane/EtOAc, 3:1 → 1:6) to give 2-[1-(1-*tert*-butoxycarbonyl-piperidin-4-yl)-ethyl]-malonic acid diethyl ester (0.39 g, 54 %).

(d) 2-[1-(1-*tert*-Butoxycarbonyl-piperidin-4-yl)-ethyl]-malonic acid monoethyl ester
A solution of KOH (84 mg, 1.16 mmol) in EtOH (2 mL) was added dropwise to a solution of 2-[1-(1-*tert*-butoxycarbonyl-piperidin-4-yl)-ethyl]-malonic acid diethyl ester (0.39 g, 1.01 mmol) in methylene chloride (4 mL) and EtOH (10 mL) at 0°C. The resulting mixture was stirred at room temperature over night. EtOAc was added and the mixture was washed with 0.5 M HCl and brine, dried and concentrated under reduced pressure to give 416 mg of crude 2-[1-(1-*tert*-butoxycarbonyl-piperidin-4-yl)-ethyl]-malonic acid monoethyl ester.

(e) 4-(2-Ethoxycarbonyl-1-methyl-allyl)-piperidine-1-carboxylic acid *tert*-butyl ester
Formaldehyde (132 mg, 1.65 mmol, 37 % in water) was added to a solution of of crude 2-[1-(1-*tert*-butoxycarbonyl-piperidin-4-yl)-ethyl]-malonic acid monoethyl ester (416 mg) in methylene chloride (2 mL) at 0°C. Diethylamine (153 µL, 1.47 mmol) was added dropwise and the mixture was stirred at room temperature over night. EtOAc was added and the mixture was washed with water and saturated $NaHCO_3$, dried and concentrated under reduced pressure. The crude was purified by flash chromatography (toluene/EtOAc, 3:1) to

give 4-(2-ethoxycarbonyl-1-methyl-allyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.18 g, 49 % over two steps).

(f) 4-(3-Acetylsulfanyl-2-ethoxycarbonyl-1-methyl-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester

TEA (86 µL, 0.617 mmol) was added to a solution of 4-(2-ethoxycarbonyl-1-methyl-allyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.18 g, 0.59 mmol) in thioacetic acid (2 mL) at 0°C. After stirring for 6 h more thioacetic acid (2 mL) was added and the mixture was stirred at 45°C over night. EtOAc was added and the mixture was washed with water, saturated $NaHCO_3$ and brine, dried and concentrated under reduced pressure. . The crude was purified by flash chromatography (toluene/EtOAc, 5:1 → 1:1) to slightly unpure 4-(3-acetylsulfanyl-2-ethoxycarbonyl-1-methyl-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.17 g, 75 %).

(g) 2-Acetylsulfanylmethyl-3-piperidin-4-yl-butyric acid ethyl ester

TFA (2 mL) was added to a solution of 4-(3-acetylsulfanyl-2-ethoxycarbonyl-1-methyl-propyl)-piperidine-1-carboxylic acid *tert*-butyl ester (0.17 g, 0.439 mmol) in methylene chloride (10 mL). The reaction was stirred for 90 min and concentrated under reduced pressure. The crude product was purified using HPLC (10 → 50 % acetonitrile in water, 0.1 % TFA) to give 2-acetylsulfanylmethyl-3-piperidin-4-yl-butyric acid ethyl ester (101 mg, 54 %) as the TFA salt.

(h) 2-Mercaptomethyl-3-piperidin-4-yl-butyric acid

Conc. hydrochloric acid (4 mL) was added to 2-acetylsulfanylmethyl-3-piperidin-4-yl-butyric acid ethyl ester TFA salt (0.101 g, 0.252 mmol) under argon. The reaction was heated to reflux for 5 h and then concentrated under reduced pressure to give a diastereomeric mixture of the title compound (73.7 mg) as the hydrochloride salt.

^{1}H NMR (500 MHz, CD_3OD) for the major diastereomer: δ 1.10 (d, 3H), 1.36-1.58 (m, 2H), 1.71-1.78 (m, 2H). 1.93-1.99 (m, 1H), 2.05-2.11 (m, 1H), 2.60-2.66 (m, 2H), 2.80-2.86 (m, 1H), 2.94-3.02 (m, 2H), 3.38-3.45 (M, 2H).

MS (+) 218 (M+1).

Example 17

3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-butyric acid

(a) (5-formyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester

(5-hydroxymethyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (7.00 g, 31.2 mmol) was dissolved in dry DMSO (50 mL) and the reaction flask immersed in a waterbath at 15°C. TEA (13.1 ml, 94.0 mmol) was added, followed by sulfur trioxide pyridine complex (15.0 g, 94.0 mmol) in portions. The reaction mixture was stirred for further 45 min and poured onto crushed ice. The product was extracted with diethyl ether and the combined organic extracts were washed with brine, dried and concentrated under reduced pressure. Recrystallisation from hexane/CH_2Cl_2 afforded (5-formyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (5.40 g, 78 %) as white crystals.

(b) 2-(6-*tert*-Butoxycarbonylamino-pyridin-3-ylmethylene)-malonic acid diethyl ester

To a solution of diethyl malonate (710 µL, 4.7 mmol) and (5-formyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (1.04 g, 4.7 mmol) in methylene chloride/DMF (1:1, 5 mL) was added piperidine (46 µL, 0.47 mmol) and acetic acid (27 µL, 0.47 mmol). The reaction mixture was stirred for 72 h at room temperature and then for 16 h at 45°C. Heptane was added slowly to give 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethylene)-malonic acid diethyl ester (0.69 g, 40 %) as grey crystals.

(c) 2-[1-(6-*tert*-Butoxycarbonylamino-pyridin-3-yl)-ethyl]-malonic acid diethyl ester

MeLi (6.5 mL, 10.4 mmol, 1.6 M in diethyl ether) was added dropwise to a slurry of CuI (0.9 g, 4.73 mmol) in THF (28 mL) at -78°C under argon. The reaction mixture was stirred for 30 min. A solution of 2-(6-*tert*-butoxycarbonylamino-pyridin-3-ylmethylene)-malonic acid diethyl ester (0.84 g, 2.3 mmol) in THF (7 mL) was added dropwise and the reaction was stirred for 180 min at -78°C. Saturated aqueous NH_4OH was added dropwise and the mixture was extracted with EtOAc. The organic phase was washed with saturated aqueous NH_4OH and NaCl, dried and concentrated under reduced pressure. Flash chromatography (toluene/EtOAc, 3:1 → 1:6) gave 2-[1-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-ethyl]-malonic acid diethyl ester (0.723 g, 82.4 %) as a white solid.

(d) 2-[1-(6-*tert*-Butoxycarbonylamino-pyridin-3-yl)-ethyl]-malonic acid monoethyl ester

A solution of KOH (113.6 mg, 2.04 mmol) in EtOH (2 mL) was added dropwise to a solution of 2-[1-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-ethyl]-malonic acid diethyl ester (0.7 g, 1.84 mmol) in methylene chloride (4 mL) and EtOH (10 mL) at 0°C under argon and the reaction mixture was stirred over night. 1M KOH (100 mL) was added and the mixture was washed with methylene chloride. The aqueous phase was acidified to pH 2 using 2 M HCl and extracted with EtOAc. The organic phase was dried and concentrated under reduced pressure to give the crude 2-[1-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-ethyl]-malonic acid monoethyl ester (423 mg, 65 %).

(e) 2-[1-(6-*tert*-Butoxycarbonylamino-pyridin-3-yl)-ethyl]-acrylic acid ethyl ester

Diethylamine (0.153 mL, 1.473 mmol) was added to a solution of 2-[1-(6-*tert*-butoxy-carbonylamino-pyridin-3-yl)-ethyl]-malonic acid monoethyl ester (423 mg, 1.2 mmol) and formaldehyde (132 mg, 1.626 mmol, 36 % in water) in methylene chloride (2 mL) at 0°C under argon. The mixture was stirred at room temperature over night. EtOAc was added and the solution was washed with water, $NaHCO_3$ and brine, dried and concentrated under reduced pressure. Flash chromatography (toluene/EtOAc, 3:1) gave 2-[1-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-ethyl]-acrylic acid ethyl ester (158 mg, 41 %).

(f) 2-Acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-butyric acid ethyl ester

TEA (0.076 mL, 0.542 mmol) was added to a solution of 2-[1-(6-*tert*-butoxycarbonyl-amino-pyridin-3-yl)-ethyl]-acrylic acid ethyl ester (158 mg, 0.493 mmol) in thioacetic acid (2 mL) at 0°C under argon. The mixture was stirred at 45°C over night. EtOAc was added and the solution was washed with $NaHCO_3$ and brine, dried and concentrated under reduced pressure. Flash chromatography (toluene/EtOAc, 5:1 → 1:1) gave slightly unpure 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonylamino-pyridin-3-yl)-butyric acid ethyl ester (178 mg, 91 %).

(g) 2-Acetylsulfanylmethyl-3-(6-amino-pyridin-3-yl)-butyric acid ethyl ester

TFA (2 mL) was added to a solution of 2-acetylsulfanylmethyl-3-(6-*tert*-butoxycarbonyl-amino-pyridin-3-yl)-butyric acid ethyl ester (178 mg, 0.449 mmol) in methylene chloride

(2 mL). The mixture was stirred for 60 min and concentrated under reduced pressure. Flash chromatography (toluene/ EtOAc, 1:6) gave unpure 2-acetylsulfanylmethyl-3-(6-amino-pyridin-3-yl)-butyric acid ethyl ester (176 mg, 95 %). Further purification by HPLC (10 → 70 % acetonitrile in water, 0.1 % TFA) gave 2-acetylsulfanylmethyl-3-(6-amino-pyridin-3-yl)-butyric acid ethyl ester (104 mg, 56 %) as the TFA salt.

(h) 3-(6-Amino-pyridin-3-yl)-2-mercaptomethyl-butyric acid

Conc. hydrochloric acid (4 mL) was added to 2-acetylsulfanylmethyl-3-(6-amino-pyridin-3-yl)-butyric acid ethyl ester (104 mg, 0.253 mmol) under argon. The reaction was heated to reflux for 5 h and then concentrated under reduced pressure to give a diastereomeric mixture of the title compound (61 mg, 92 %) as the hydrochloride salt.

^{1}H NMR (500 MHz, D_2O) for the major diastereomer: δ 1.26 (d, 3H), 2.49-2.53 (m, 2H), 2.64-2.77 (m, 1H), 2.95-3.02 (m, 1H), 7.02 (d, 1H), 7.69 (d, 1H), 7.88 (m, 1H).

MS (+) 227 (M+1).

Example 18

3-(6-Amino-2-methyl-pyridin-3-yl)-2-mercaptomethyl-propionic acid

(a) (5-Bromo-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester

5-Bromo-6-methyl-pyridin-2-ylamine (25.0 g, 133.7 mmol) in THF/tert-butanol (1:10, 550 mL) was treated with di-tert-butyl dicarbonate (39.3 g, 180.0 mmol) and DMAP (2.40 g, 19.6 mmol). The reaction mixture was stirred for 4 h at 40°C and concentrated under reduced pressure. Flash chromatography (methylene chloride) gave (5-bromo-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (17.0 g, 44 %).

(b) [5-(*tert*-Butyl-dimethyl-silanyloxymethyl)-6-methyl-pyridin-2-yl]-carbamic acid *tert*-butyl ester

A solution of (5-bromo-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (27.5 g, 95.8 mmol), *tert*-butyl-dimethyl-tributylstannanylmethoxy-silane (43.5 g, 100.2 mmol), and bis(triphenylphosphine)palladium(II) dichloride (1.00 g, 1.40 mmol) in 1,2-dichloroethane (350 mL) was stirred at reflux for 48 h. Additional bis(triphenylphosphine)palladium(II) dichloride (1.00 g, 1.40 mmol) was added every 12 h. The mixture was cooled to 0°C and

diethyl ether (300 mL) was added followed by saturated aqueous potassium fluoride (100 mL). The mixture was stirred vigourously for 60 min and filtered. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography ($MeOH/CH_2Cl_2$, 1:99) gave [5-(*tert*-butyl-dimethyl-silanyloxymethyl)-6-methyl-pyridin-2-yl]-carbamic acid *tert*-butyl ester (15 g, 47 %).

(c) (5-Hydroxymethyl-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester

Tetrabutylammonium fluoride (19.6 g, 62.4 mmol) was added to a solution of [5-(*tert*-butyl-dimethyl-silanyloxymethyl)-6-methyl-pyridin-2-yl]-carbamic acid *tert*-butyl ester (10.5 g, 31.23 mmol) in THF (100 mL) and stirred at room temperature overnight. Water was added and the product extracted with chloroform. The organic phase was dried and concentrated under reduced pressure. Flash chromatography ($MeOH/CH_2Cl_2$, 2.5:77.5) gave (5-hydroxymethyl-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (6.0 g , 81 %).

(d) 5-Bromomethyl-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-pyrimidin

Triphenylphosphine (9.83 g, 37.5 mmol) and CBr_4 (17.7 g, 53.5 mmol) was added to a solution of (5-hydroxymethyl-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (8.50 g, 35.7 mmol) in dichloromethane (30 mL) at 0°C. The reaction mixture was stirred for 3 h at room temperature and was then diluted with dichloromethane. The organic phase was washed with water, dried and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2) gave 5-bromomethyl-2-[*N,N*-bis(*tert*-butoxycarbonyl)amino]-pyrimidin (4.05 g, 38 %).

(e) 2-(6-*tert*-Butoxycarbonylamino-2-methyl-pyridin-3-ylmethyl)-malonic acid diethyl ester

A solution of diethyl malonate (1.21 mL, 7.97 mmol) in DMF (2 mL) was added dropwise to a suspention of NaH (348 mg, 7.97 mmol, 55 % in mineral oil) in DMF (5 mL) at 0 °C under argon. The reaction mixture was stirred for 45 min and a solution of (5-bromo-methyl-6-methyl-pyridin-2-yl)-carbamic acid *tert*-butyl ester (2.0 g, 6.64 mmol) in DMF (5 mL) was added dropwise. The mixture was stirred over night (0°C → 20°C). EtOAc was added and the solution was washed with water and brine, dried and concentrated under